Point of VIEW

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Reverse Interactomics: From Peptides to Proteins and to Functions

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ABSTRACT Identifying associated partners is critical to understanding the potential function and upstream and downstream pathways of the molecule being studied. The Src homology 2 (SH2) domain is a binding module for peptides containing a phosphotyrosine residue, which is a post-translational modification that is heavily involved in signal transduction. A paper in this issue shows how "reverse interactomics" can be exploited to identify binding partners of the SH2 domain of tensin.

Following the genome era, interactomics has become a new discipline that studies the entire protein–protein interaction network in cells. Protein–protein interactions regulate many biological events. Identification of binding partners is crucial for understanding the biological processes regulated by your favorite molecules. Experimental approaches usually involve affinity purification or the yeast-two-hybrid system (*1*–*3*). Although they are used with success in some cases, these techniques have limitations. For example, affinity purification is low-throughput and usually restricted to the identification of the most-abundant and high-affinity binding proteins. The yeast-two-hybrid system is relatively high-throughput but is often associated with high false-positive and false-negative results. Also, it cannot be used to detect interactions that rely on post-translational modifications, such as tyrosine phosphorylation, which do not normally occur in yeast cells.

In a paper in this issue, Wavreille and Pei (*4*) took a "reverse interactomics" approach (Figure 1) to identify binding partners for the Src homology 2 (SH2) domain of tensin, a focal adhesion molecule that regulates the actin cytoskeleton and mediates processes associated with cell adhesion, migration, and tumor-suppression activity (*5*) (Figure 2). The SH2 domain is the most prevalent protein binding module that recognizes phosphotyrosine (pY). It is a highly conserved ~100 amino acid residue domain that is found in a wide variety of proteins (*6*). The SH2-containing proteins often possess one or more of other protein modules, such as catalytic domains and other binding domains, and play various roles in protein tyrosine kinase signaling pathways.

In their efforts to identify new associated proteins, Wavreille and Pei (*4*) nicely combine tools from chemistry, bioinformatics, and biochemistry. These technologies have been established for years. For example, the combinatorial library (one bead–one compound) was developed >15 yr ago (*7*–*9*) and has been successfully used to identify peptide substrates and inhibitors of protein kinases and peptide ligands for cell surface receptors (*10*). This method synthesizes millions of random peptides (*e.g.*, tyrosine-phosphorylated peptides, as is the case in the Wavreille and Pei (*4*) paper) such that each bead displays only one peptide. Bead libraries are screened, and positive beads are isolated for sequence analysis. Once the "consensus sequences" are identified, the sequences are analyzed against more complete databases using the BLAST algorithm. The molecules containing the consensus sequences are then selected on the basis of their known function, and, as is the case in the Wavreille and Pei (*4*) paper, subcellular localization. *In vitro* binding assays are used to validate the interactions.

Three classes of consensus sequences for tensin's SH2 domain are identified in this paper. Class I (pY[E/D]N[V/M/Y/L]) corresponds to the sequence identified 10 yr ago by Auger *et al.* (*11*), validating the com-

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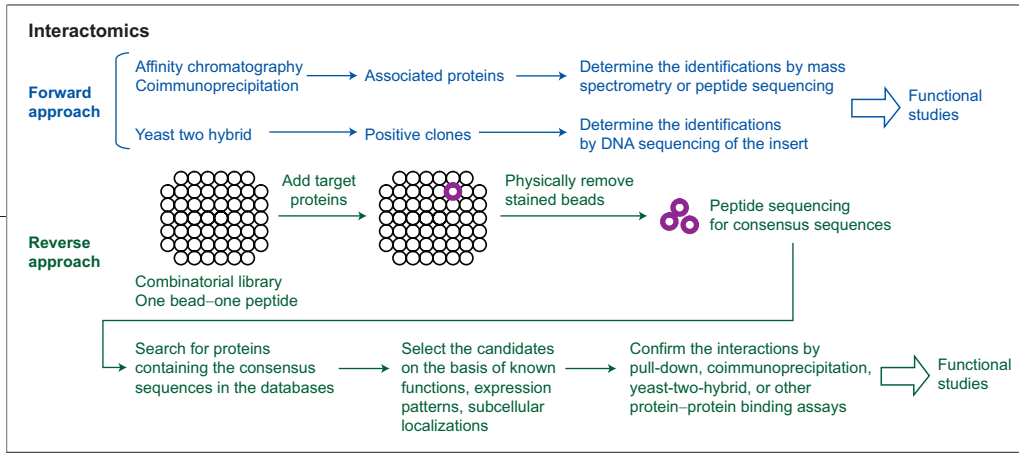

Figure 1. Approaches in interactomics. Reverse interactomics is an approach to identifying the binding partners of a protein that proceeds in the opposite direction of (forward) interactomics, which usually involved direct identification of associated proteins by techniques such as affinity chromatography, coimmunoprecipitation, or the yeast-two-hybrid system, followed by sequence analysis. As an example of how reverse interactomics seeks to find the consensus binding sequence(s) for a molecule, one can first add a recombinant SH2 domain of tensin to a combinatorial library. Development of color indicates that some beads are interacting with the SH2 domain. These beads are physically removed, and the bound peptides are sequenced to derive the consensus binding sequences. Candidate proteins containing the consensus binding sequences in the genome are identified. Subsequent analysis confirms whether these candidates interact with the target protein.

binatorial library approach.The class I sequence is used to probe databases and identify 29 potential tensin-binding proteins, which are further selected on the basis of their subcellular localization (because tensin is a membrane-associated, focal adhesion molecule). Among them, p130Cas has previously been reported to interact with tensin (*12*). They further confirm the interactions between tensin and two molecules, phosphoinositide-dependent kinase-1 (PDK-1) and downstream of tyrosine kinase 2 (Dok-2) , using pull-down and coimmunoprecipitation assays. Nonetheless, these findings raise many new questions. Are the potential binding sites in the candidate molecules tyrosine-phosphorylated in cells, and if so, when? Under which cellular conditions does tensin bind to PDK-1, Dok-2, and others? How do these interactions affect tensin's function, and how does this relate to its physiological roles? The biological relevance of these interactions remains to be explored.

On the basis of the binding consensus sequences, the authors have designed a pY peptide to function as a tensin SH2 domain inhibitor. When introduced into cells, it leads to ~30% more cells with disrupted actin filaments compared with those of control nonphosphorylated peptide treatment. These data imply that the pY peptides bind to the SH2 domain of tensin and somehow affect the organization of the actin cytoskeleton. This offers a potential pharmaceutical application to modulate tyrosine kinase signaling in human disease.

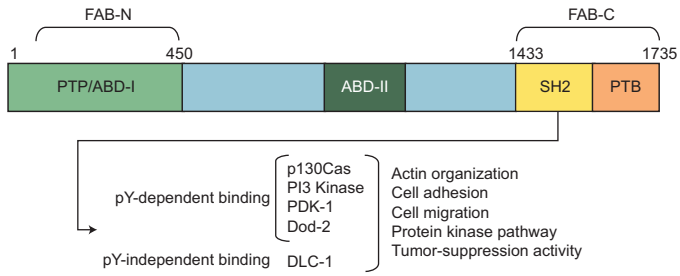

Figure 2. Domain functions of tensin. The N-terminus contains the actin binding domain (ABD) I and phosphatase and tensin homologue (PTEN)-related protein tyrosine phosphatase (PTP) domain. This region also contains a focal adhesion binding (FAB) domain. ABD II in the center region of tensin retards the actin polymerization rate. The C-terminus contains an SH2 domain, pY binding (PTB) domain, and the second FAB site. The PTB domain interacts with the NPXY sites in β integrins. Most of the associated proteins that bind to the SH2 domain of tensin require phosphorylation on tyrosine residues. The interaction between DLC-1 and tensin is independent of tyrosine phosphorylation. The SH2 binding to these molecules somehow regulates actin organization, cell adhesion, migration, and tumor-suppression activity.

The specificity is a critical issue for inhibition purposes. Many binding consensus sequences in SH2 domains are shared by other molecules involved in different signaling pathways (*6*). Designing a binding peptide that only inhibits tensin but not Src, which also recognizes pYDNL, for example, is very challenging! Furthermore, tensin represents a gene family with four members (tensin1, tensin2, tensin3, and cten) (*5*). They all share the highly conserved SH2 domain (80–90% similarity in amino acid sequences). Whether a peptide can be specific for tensin1 but not other tensins is questionable.

Notably, although SH2 domains are known to interact with tyrosine-phosphorylated peptides, cases have been reported in which some SH2 domains can bind to non-tyrosine-phosphorylated peptides, and tensin is one of them (*13, 14*). Scientists have shown that all tensin SH2 domains bind to DLC-1 (deleted in liver cancer 1), a tumor suppressor, independently of tyrosine phosphorylation of DLC-1 (*14*). This interaction is essential for DLC-1's focal adhesion localization, which in turn is critical for its tumor-suppression activity (*14*). Therefore, this pY-independent binding is something to keep in mind when one is screening binding ligands for SH2 domains and interpreting results.

Overall, the reverse interactomics approaches will greatly increase scientists' ability to identify potential binding partners. It is critical to carefully evaluate and analyze the candidates identified from data mining experiments and to validate the interactions *in vitro* and *in vivo*. Finally, elucidation of the biological relevance of the interaction is the most important issue of all!

The work presented by Wavreille and Pei (*4*) increases our insight of the signal transduction pathways mediated by the SH2 domain of tensin and is an excellent example of the integration of the fields of chemistry, bioinformatics, and biology.

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